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IN RE APPLICATION OF: Paulo C. G. FERREIRA, et al.
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FOR: PLANTS HAVING CHANGED DEVELOPMENT AND A METHOD FOR MAKING THE SAME

REQUEST FOR PRIORITY UNDER 35 U.S.C. 119 AND THE INTERNATIONAL CONVENTION

Commissioner for Patents
Alexandria, Virginia 22313

Sir:

In the matter of the above-identified application for patent, notice is hereby given that the applicant claims as priority:

COUNTRY	APPLICATION NO	DAY/MONTH/YEAR
EPC	PCT/EP02/10265	05 September 2002

Certified copies of the corresponding Convention application(s) were submitted to the International Bureau in PCT Application No. PCT/EP03/10087. Receipt of the certified copy(s) by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.

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Bescheinigung

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten internationalen Patentanmeldung überein.

Certificate

The attached documents are exact copies of the international patent application described on the following page, as originally filed.

Attestation

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet international spécifiée à la page suivante.

Den Haag, den
The Hague,
La Haye, le 16. 10. 2003

Der Präsident des Europäischen Patentamts
Im Auftrag
For the President of the European Patent Office
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Patentanmeldung Nr.
Patent application no.
Demande de brevet n° PCT/EP 02/10265

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

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Plants having modified development and a method for making the same

Background

The regulation of the cell cycle in plants is poorly understood. Most of the knowledge regarding the regulation of DNA replication, also known as the S-phase of the cell cycle regulation originates from experimental data obtained in yeast and mammalian cells. However, the importance to understand the cell cycle regulation in plant cells has become increasingly important in agriculture, e.g. to ensure survivability of plants in unfavourable growth conditions, or to obtain resistance or tolerance against pathogens, and/or to improve economically important agronomic characteristics such as yield.

Detailed description

The inventors of the present application previously described the isolation of the cdc27A gene (SEQ ID NO 1), encoding a protein as in SEQ ID NO 2) and a splice variant thereof (WO 01/02430), as a gene involved in DNA synthesis.

Surprisingly it has now being found that introduction of a cdcd27A in Tobacco plants, gives rise to plants having altered developmental characteristics. In particular it was unexpected that the transgenic plants showed increased rate of development. This beneficial effect was in balance with the overall architecture and shape of the plant and the acceleration of the plant development resulted in healthy adult plants that reach the flowering stage earlier than control plants. Additionally, the adult plants appeared to grow larger. The effects of the introduction of a transgene becomes progressively more pronounced in the later stages of development of the plant. It was further surprising to find the same plants having an increased of the number of flowers and/or increased number of seeds.

These technical features are particularly favourable when commercially producing plants, because these technical features contribute to increase yield.

It has now being found that introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof gives rise to Plants having altered development.

Therefore according to the present invention there is provided a method for altering plant development, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof.

The effects of introducing the expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof can be any one or more of the following: enhanced transcript level, enhanced protein level, enhanced expression

of the encoded gene or decrease of expression of the native gene or enhanced activity of the encoded protein.

It has now being shown that by introducing a cdcd27a gene into a plant, it is possible to alter plant development and more particularly, alter one or more of the following phenotypic characteristics: faster development, early flowering, more flowers, more seeds, bigger leaves and increased height.

Accordingly a particular embodiment of the invention relates to a method as described above wherein said altering plant development comprises increasing the rate of development of a plant or plant part relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altering plant development comprises altered flowering characteristics relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altering flowering characteristics comprises altering the period of time to reach flowering relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altered period of time to reach flowering comprises early flowering relative to corresponding wild-type plants.

Plant development also includes organ formation. Accordingly, altering plant development includes altering the number of organs for example the number of flowers and/ or the number of seeds.

Accordingly, a preferred embodiment the present invention relates to a method as described above wherein said altering flowering characteristics comprises increasing the number of flowers relative to corresponding wild-type plants.

Accordingly, a preferred embodiment the present invention relates to a method as described above, wherein said altering plant development comprises increasing the number of seeds relative to corresponding wild-type plants.

Another aspect of the invention is the use of a nucleic acid encoding a cdcd27a protein. More particularly, the inventors cloned the cdcd27A encoding gene in an expression vector that is expressible in plants.

Accordingly, the invention also embodies a method as described above, wherein said plant-expressible vector comprises:

a) A nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof;

b) A regulatory sequence capable of driving expression of said nucleic acid sequence of a); and

c) Optionally, a terminator sequence.

According to a preferred embodiment of the present invention, a nucleic acid sequence encoding a cdc27A protein is a nucleic acid sequence encoding the cdcd27A1 protein from *Arabidopsis thaliana*

Further preferable, the cdc27A protein is cdc27A1 as depicted in SEQ ID NO 2

Alternatively, said cdcd27A1 protein is encoded by SEQ ID NO 1.

The present invention further relates to plants obtained by the methods of the present invention and showing one or more of the following specific phenotypes: faster development, early flowering, more flowers, more seeds, bigger leaves and increased height.

Another aspect of the invention is the transgenic plant that is obtained by the present invention. Accordingly, another embodiment of the present invention is a transgenic plant obtained by any method as described above, said plant having altered plant development.

Also, another embodiment of the invention relates to the ancestors or progeny of a plant as described above, said ancestor or progeny having altered plant development relative to corresponding wild type plants

Definitions

"altering development" as described herein encompasses increased (faster or accelerated) rate of development or a decreased rate of development or induced development at any one or more developmental stages of the life cycle of a plant.

Increased development encompasses an increase in the rate of progression through one or more, or part of one or more, of the different stages of the life cycle of the plant. The life cycle of a plant encompasses any stage from a dormant seed to a senescing plant and any stage in between. Further more accelerated development also encompasses stages leading up to seed formation.

One can envisage a situation where a plant can grow faster, but where the plant does not reach a certain developmental stage.

According to the present invention, the term "altered flowering characteristics" encompasses one or more of the following: altered period of time to reach flowering, altered number of flowers, altered length flowering period, each relative to corresponding wild type plants

According to the invention, the "gene" or the "polypeptide" may be the wild type, i.e. the native or endogenic nucleic acid or polypeptide which expression is modified. Alternatively, the gene may be a heterologous nucleic acid derived from the same or another species and be introduced as a transgene for example by transformation. This transgene may be substantially modified from its native form in composition and/or genomic environment through deliberate

human manipulation. Also expression of the native genes can be modified introduction in the plant of regulatory sequences that alter the expression of the native gene.

One way of modifying the expression of cdcd27A according to the invention relates to a method comprising the stable integration into the genome of a plant or in specific plant cells or tissues of said plant of an expressible gene encoding a plant cdc27, a homologue or a derivative thereof or an enzymatically active fragment thereof.

The term "overexpression" should be understood as "ectopic expression". "Ectopic expression" or "ectopic overexpression" of a gene or a protein refers to expression patterns and/or expression levels of said gene or protein normally not occurring under natural conditions. Ectopic expression can be achieved in a number of ways including operably linking of a coding sequence encoding said protein to an isolated homologous or heterologous promoter in order to create a chimeric gene and/or operably linking said coding sequence to its own isolated promoter (i.e. the unisolated promoter naturally driving expression of said protein) in order to create a recombinant gene duplication or gene multiplication effect.

According to at least one preferred embodiment of the invention, enhanced or increased expression of said nucleic acid is envisaged. Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and are for example overexpression driven by a strong promoter, the use of transcription enhancers or translation enhancers. Examples of decreasing expression are also well documented in the art and are for example: downregulation of expression by anti-sense techniques, gene silencing etc.

The term "overexpressing" in the present invention also means modifying the activity of the gene product, more particularly in the present invention the cdc27A protein in the host cell. This can be achieved for example by respectively inhibiting or stimulating the control elements that drive the expression of the native gene or of the transgene. Also modifying the activity of the gene product, the polypeptide, can furthermore be achieved by administering or exposing cells, tissues, organs or organisms to, an interacting protein or an inhibitor or activator of said gene product. In the context of the present invention, such inhibitors or activators can also effect their activity against the cdcd27A protein in order to obtain the effects as claimed in the present invention.

Also modifying, increasing, the activity of the gene product, the polypeptide, can furthermore be achieved by administering or exposing cells, tissues, organs or organisms to, a preparation of said gene product, so that it can exert its functions in said exposed cells or tissues. In the context of the present invention, the cells are exposed to protein samples of cdcd27A protein.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant cell" also encompasses suspension cultures, embryos,

meristematic regions, callus tissue, leaves, seeds, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

Advantageously, the method according to the present invention is applicable to algae, fungi, angiosperms and gymnosperms, both monocotyledonous and dicotyledonous plants. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp.,Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp.,Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata; Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp.Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays*, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot,

cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, trees and algae amongst others. According to a preferred feature of the present invention, the plant is a monocotyledonous plant, further preferably a crop plant such as rice, maize, wheat, barley, soybean, sunflower, canola, alfalfa, millet, barley, rapeseed and cotton.

A "vector" as used herein refers to a nucleic acid used for transfection or transformation of a host cell and into which a nucleic acid sequence can be inserted. Expression vectors allow transcription and/or translation of a nucleic acid inserted therein. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. The term "expressible" or "plant-expressible" relates to the presence of control sequences which promote adequate expression of genes and/or proper translation of said sequences into a protein.

The terms "gene(s)", "polynucleotide(s)", "nucleic acid(s)", "nucleotide sequence(s)", "nucleic acid sequences" or "nucleic acid molecules", are used interchangeably herein and encompass a polymeric form of a deoxyribonucleotide or a ribonucleotide polymer of any length, either double- or single-stranded, or analogues thereof, that have the essential characteristic of a natural ribonucleotide in that they can hybridise to nucleic acids in a manner similar to naturally occurring polynucleotides.

A great variety of modifications, which are known in the art, can be made to DNA and RNA and can serve many useful purposes. Such modifications include methylation, 'caps' and substitution of one or more of the naturally occurring nucleotides with an analogue.

"Sense strand" refers to a DNA strand that is homologous to an mRNA transcript thereof; "anti-sense strand" refers to an inverted sequence which is complementary to the "sense strand".

"Encoding" or "encodes", with respect to a specified nucleotide sequence, refers to the information for translation into a specified protein. A nucleic acid encoding a protein may contain non-translated sequences such as 5' and 3' untranslated regions (5' and 3' UTR) and introns or it may lack intron sequences, such as in cDNAs.

An "open reading frame" or "(ORF)" is defined as a nucleotide sequence that encodes a polypeptide. The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the 'universal' genetic code but variants of this universal code exist (see for example Proc. Natl. Acad. Sci. U.S.A 82: 2306-2309 (1985)). The boundaries of the coding sequence are determined by a translation start codon at the 5'-end and a translation stop codon at the 3'-terminus. Because of the degeneracy of the genetic code, a large number of nucleic acids can encode any given protein. As such, substantially divergent nucleic acid sequences can be designed to effect expression of essentially the same protein in different hosts. Conversely, genes and coding sequences essentially encoding the same protein isolated from different sources can consist of substantially different nucleic acid sequences.

Methods for the search and identification of homologues of CDC27A, would be well within the realm of a person skilled in the art. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information. The cdc27A homologue from *Arabidopsis* identified above was identified using BLAST default parameters.

"Homologues" encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or additions relative to the protein in question and having similar biological and functional activity as an unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company). The homologues useful in the method according to the invention most preferably have a degree of sequence identity or similarity (functional identity) to, for example, the unmodified CDC27A protein, wherein said degree of sequence identity or similarity is typically at least 50%, more typically at least 60%, preferably at least 70%, further preferably at least 80%, most preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 99% sequence identity or similarity to an unmodified CDC27A.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "parologous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins according to the invention.

"Substitutional variants" of a protein of the invention are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions.

"Insertional variants" of a protein of the invention are those in which one or more amino acid residues are introduced into a predetermined site in said protein. Insertions can comprise

amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)$_6$-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein of the invention are characterised by the removal of one or more amino acids from the protein. Amino acid variants of a protein of the invention may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

"Derivatives" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring cdc27A protein.

"Active fragments" or "functional fragment" of CDC27A encompasses at least five contiguous amino acid residues of a CDC27A protein, which residues retain similar biological and/or functional activity to the naturally occurring CDC27A.

Also allelic variants and splice variants of the cdcd27A protein can be used for the methods of the present invention;

The expression "introducing" a plant expressible vector relates any transformation technique.

The recombinant DNA constructs for use in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The recombinant gene constructs may be inserted into vectors, which may be

commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.

Optionally, the gene of interest may be associated with a selectable marker gene. Such a marker gene encodes a trait or a phenotype which allows the selection of a plant or plant cell containing the marker. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance. Cells containing the recombinant DNA will thus be able to survive in the presence of antibiotic or herbicide concentrations that kill untransformed cells. Examples of selectable marker genes include the *bar* gene which provides resistance to the herbicide Basta; the npt gene which confers resistance to the antibiotic kanamycin; the hpt gene which confers hygromycin resistance. Visual markers, such as the Green Fluorescent Protein (GFP) may also be used as selectable markers. An entire plant may be generated from a single transformed plant cell through cell culturing techniques known to those skilled in the art.

The gene of interest is introduced into a plant by transformation. The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid, or alternatively, may be integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. Transformation methods include the use o agrobacterium, liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Preferable a preferred method is leaf disk transformation. Alternative, methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. *et al.*, 1882, Nature 296, 72-74; Negrutiu I. *et al.*, June 1987, Plant Mol. Biol. 8, 363-373); electroporation of protoplasts (Shillito R.D. *et al.*, 1985 Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A. *et al.*, 1986, Mol. Gen Genet 202, 179-185); DNA or RNA-coated particle bombardment (Klein T.M. *et al.*, 1987, Nature 327, 70) infection with (non-integrative) viruses and the like. A preferred method according to the present invention comprises the protocol according to Hiei *et al.*1994.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced

DNA may be undertaken using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The present invention also provides plants obtained by the method according to the present invention.

According to the present invention, plants are transformed with a vector comprising the sequence of interest (i.e., a nucleic acid sequence comprising CDC27A), wherein said gene is operably linked to a promoter, preferably to a constitutive.

The terms "regulatory element", "control sequence" "promoter" are all used herein interchangeably and taken in a broadest context refer to regulatory nucleic acid sequences capable of effecting expression of the sequences to which they are ligated. Encompassed by the aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a –35 box sequence and/or –10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. The terms "control sequence", "regulatory sequence", "regulatory element" and "promoter" are used interchangeably herein. The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Examples

The present invention will now be described with reference to the following examples, which are by way of illustration alone.

Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York; or in Volumes 1 and 2 of Ausubel *et al.* (1984), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

In a particular example of the present invention, said cdc27A gene is isolated and cloned in a plant expressable vector carrying the CaMV35S promoter.

In a particular example of the present invention, the CaMV35S::cdcd27A construct is subsequently transformed and stably integrated in the genome of Tobacco.

In a particular example of the present invention the cdcd27A gene is a dcd27A gene of *Arabidopsis thaliana* or is a gene as presented in SEQ ID NO 1, encoding the cdc27A protein as presented in SEQ ID NO 2. Alternatively other homologues, allelic variants and splice variants can be used for the methods of the present invention.

Example of cloning cdcd27A1:

To express constitutively the Arabidopsis CDC27a cDNA in transgenic plants, PCR mutagenesis has been carried out to introduce restriction sites in the full-length cDNA. Oligonucleotides containing NcoI and BamHI restriction sites were used in a PCR reaction, the resulting fragment was restricted with the 2 enzymes (NcoI and BamHI). The CDC27a reading frame was ligated in the PH35S (Hemerly et al. EMBO J. 14, 3925-3936), in the same sites, in front of the 35S promoter, and with the NOS terminator. The resulting plasmid was digested with EcoRI, filled in with Klenow enzyme, and then cut with SalI to release a fragment contaning the 35S promoter, the CDC27 reading frame, and the NOS terminator. This fragment was cloned in the PGSV4 plasmid in the SalI and ScaI sites. The resulting plasmid was introduced in Agrobacterium tumefasciens C58.

Tobacco plants were transformed with the resulting Agrobacterium strain. Seeds of the tobacco T1 plants were germinated in medium containing kanamycin to determine the number of copies of the transgene. Plants with a 3 to 1 relation of kanamycin resistant to susceptible seedlings were chosen to produce seeds in order to obtain homozigous plants.

Example of transformation of tobacco plants

For introduction of the gene encoding the cdcd27A protein into tobacco plants, the leaf disk method was used . This method is previously described in Horsch,R.B. et al. (1985) A simple and general method for transferring genes into plants Science 227 1229 – 1231.

Description of the Figures

The present invention will now be described with reference to the following figures in which:

Figure 1 is a schematic representation of the construct used for transforming the plants of the present invention.

Figure 2 illustrates that newly developed leaves develop faster in cdcd27A transgenic plants compared to control plants (SR1). Number 1 to 6 correspond to the leaves as they grow on the stem of the plant, meaning that leaf 1 is the leaf developed in the juvenile plant and leaf

6 being the most recently developed leaf, i.e. a leaf developed when the same plant is in a more mature stage. The data illustrate that the effects of introduction of a cdc27A gene into a plant on plant development becomes progressively more pronounced as the plant matures. The lower line designated 35S13.3/1, shows the leaves of a transgenic plant transformed with 35S::cdc27A1 and the upper line designated SR1 shows the leaves of a non transgenic control plant. These pictures illustrate that the transgenic leaves are bigger and appear earlier during the plant life cycle compared to the control plant. The results illustrate that transgenic plants have an accelerated rate of development. The results further illustrate that, at the same developmental stage, transgenic leaves are bigger relative to the leaves of control plants, indicating that the transgenic pants have increased growth.

Figure 3 illustrates transgenic plants transformed with 35S::cdc27A (positions 1 to 4) and a non transgenic control plant (position 5). The transgenic plants as seen in the illustration are taller, thereby indicating faster development. Additionally the transgenic plants flower earlier than the control plant as also illustrated in the figure. The picture shows that transgenic plants that develop faster reach an adult stage faster than the control plant without growth arrest or any apparent deformation or any apparent aberrant development during their life cycle.

Figure 4 (A to C) is a graphical illustration indicating that more recent leaves (leaves of the more mature plant, B and D) of the transgenic 35S::cdc27A1 plants develop quicker than leaves of a more juvenile transgenic plant (A and C). This is illustrated by the graphs indicating the length of the leaves number 1, 2, 6 and 7. This numbering corresponds to the leaf numbering of Figure 2. It is illustrated that the leaf length of the newly developed leaves 6 and 7 of the transgenic plant lines (1.1, 1.3, 18.1, 25, 3.2) is higher than that of control line SR1.

Figure 5 is a graphical representation illustrating that more recent leaves (i.e. of a more mature plant, B and D) of the transgenic 35S::cdc27A1 plants develop quicker than leaves of a more juvenile plant (A and C). This is illustrated by the graphs indicating the width of the leaves number 1, 2, 6 and 7. This numbering corresponds to the leaf numbering of Figure 2. It is illustrated that the leaf width of the newly developed leaves 6 and 7 of the transgenic plant lines (1.1, 1.3, 18.1, 25, 3.2) is higher than of the control line SR1.

Figure 6 is the nucleic acid sequence and protein sequence of the *Arabidopsis thaliana* cdcd27A protein.

Table 1 : Transgenic plants transformed with cdc27A flower earlier that control plants (see column flowering time). Also, transgenic plants are taller when they have reached the flowering stage compared to the non transgenic control plant (see column plant height at flowering time). From these data it is concluded that the transgenic plants show faster development, that they grow taller and that they flower early.

Table 1

Line	genotype	flowering time, mean after sowing (in days)*	Plant height mean at flowering time (cm)*	leaves number at flowering time*	Leave length/with ratio
1.1	homozygous	126,5 +- 11,13	63,8 +- 11,77	19,25 +- 1,98	1,87 +- 0,327 ***
1.3	homozygous	123,3 +- 16,66	6,3 +- 24,12	17,6 +- 3,75	1,76 +- 0,36 ***
18.8	hemizygous	124,8 +- 7,17	59,9 +- 16,70	18,2 +- 1,61	1,69 +- 0,28 ***
25 **	homozygous	138,5 +- 20,30	37,87 +- 19,98	17 +- 3,65	1,95 +- 0,27 ***
32	homozygous	127,2 +- 7,79	41,1 +- 11,11	16,8 +- 1,28	1,71 +- 0,28 ***
SR1	no transgene	147,6 +- 16,30	29 +- 4,6	17,8 +- 1,35	1,78 +- 0,13 ***

* 95%

** three of five plants

*** Mmean ± SD calculated from leave 6 of five plants with 74 days ($p < 0,05$)

Table 2: Transgenic plants transformed with 35S::cdcd27A1 have more flowers. Measurements involved five plants of each transgenic line and measurements of the control plants involved in the control line only two SR1 plants. These data illustrate that the introduction of cdcd27A in plant has an influence on plant development and more a particularly on organ formation, such as number of flowers. Furthermore it is envisaged that by using the methods of the present invention also the number of seeds is increased.

Line	1.1	1.3	18.1	25	32	SR1 *
	23,25	31,33	21,2	14	18,2	12,5

Claims

1. Method for altering plant development, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof.

2. Method according to claim 1 wherein said altering plant development comprises increasing the rate of development of a plant or plant part relative to corresponding wild-type plants.

3. Method according to claims 1 or 2, wherein said altering plant development comprises altered flowering characteristics relative to corresponding wild-type plants.

4. Method according to claims 3, wherein said altering flowering characteristics comprises altering the period of time to reach flowering relative to corresponding wild-type plants.

5. Method according to claims 4, wherein said altered period of time to reach flowering comprises early flowering relative to corresponding wild-type plants.

6. Method according to any of claims 3 to 5 wherein said altering flowering characteristics comprises increasing the number of flowers relative to corresponding wild-type plants.

7. Method according to any of claims 1 to 6, wherein said altering plant development comprises increasing the number of seeds relative to corresponding wild-type plants.

8. A method according to any of claims 1 to 7, wherein said plant-expressible vector comprises:

   a) A nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof;
   b) A regulatory sequence capable of driving expression of said nucleic acid sequence of a); and
   c) Optionally, a terminator sequence.

9. A method according to any of claims 1 to 8, wherein said cdc27A protein is cdc27A1 from *Arabidopsis thaliana*

10. A method according to any of claim 1 to 9, wherein said cdc27A protein is cdc27A1 as depicted in SEQ ID NO 2

11. Transgenic plant obtained by the method according to any of claims 1 to 10, said plant having altered plant development.

12. Ancestors or progeny of a plant according to claim 11, said ancestor or progeny having altered plant development relative to corresponding wild type plants

Abstract

Plants having modified development and a method for making the same

The present invention relates to a method for altering plant development, in particular altered flowering characteristics, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof. Plants generated according to the invention show modified development and flowering characteristics

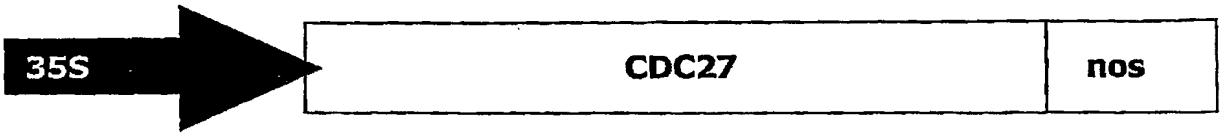


FIGURE 1

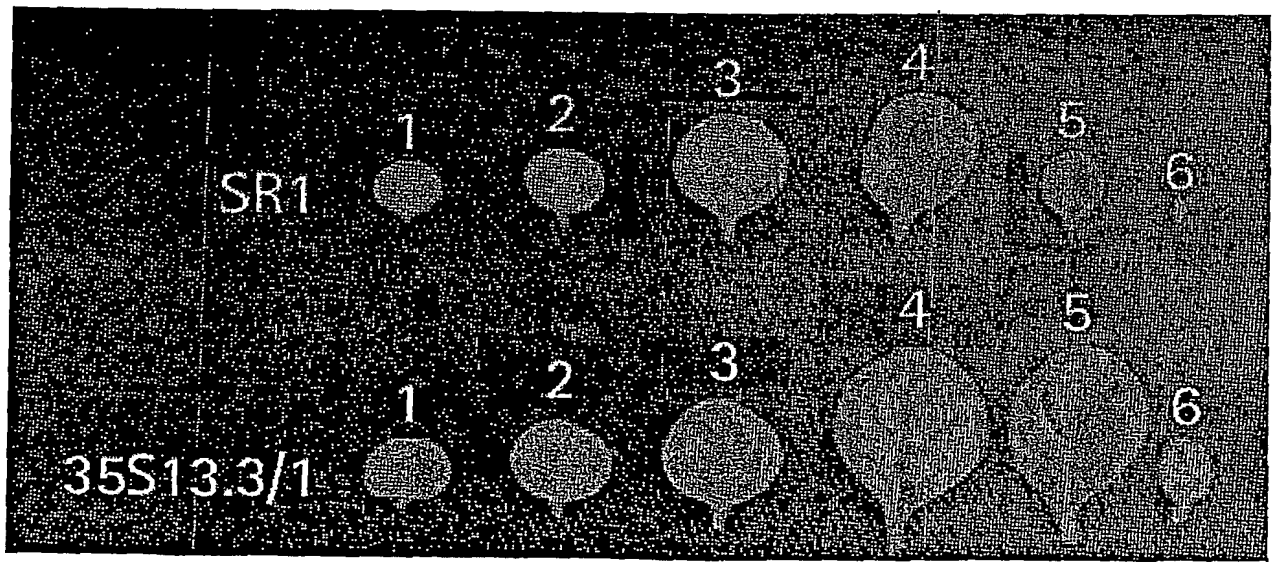
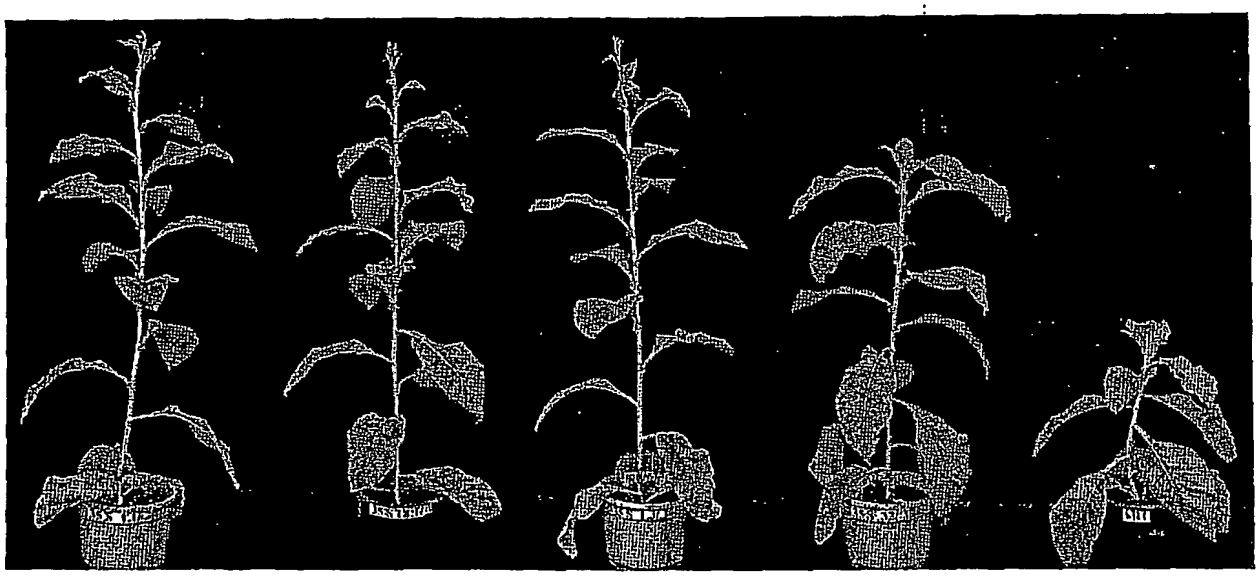


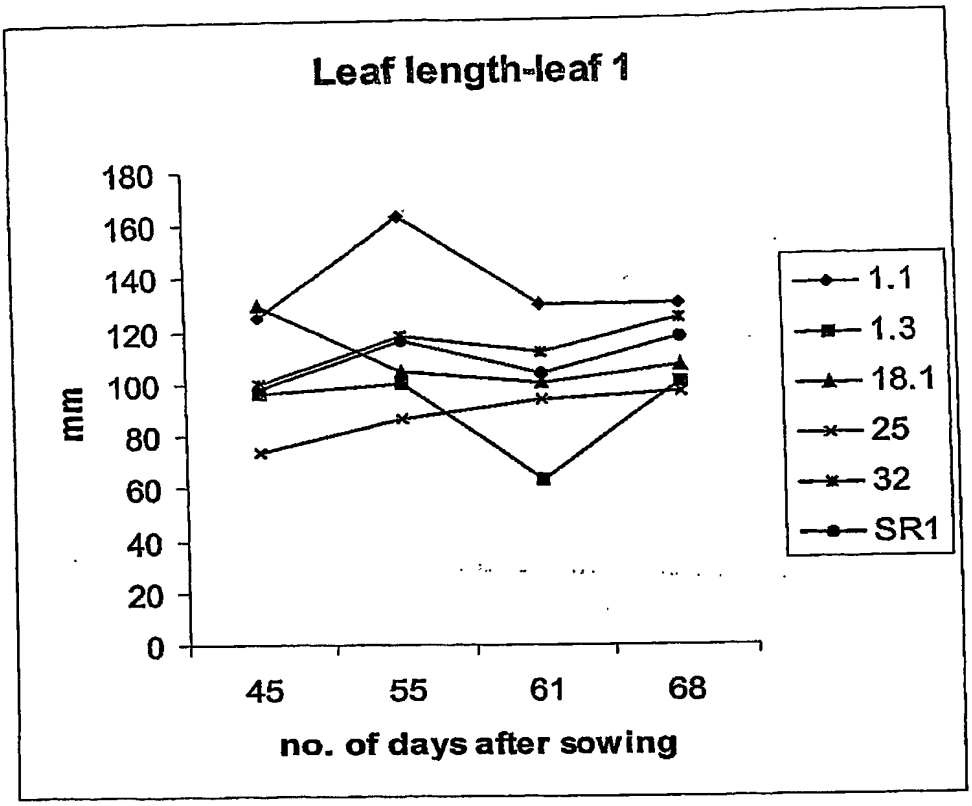
FIGURE 2



1 2 3 4 5

FIGURE 3

A



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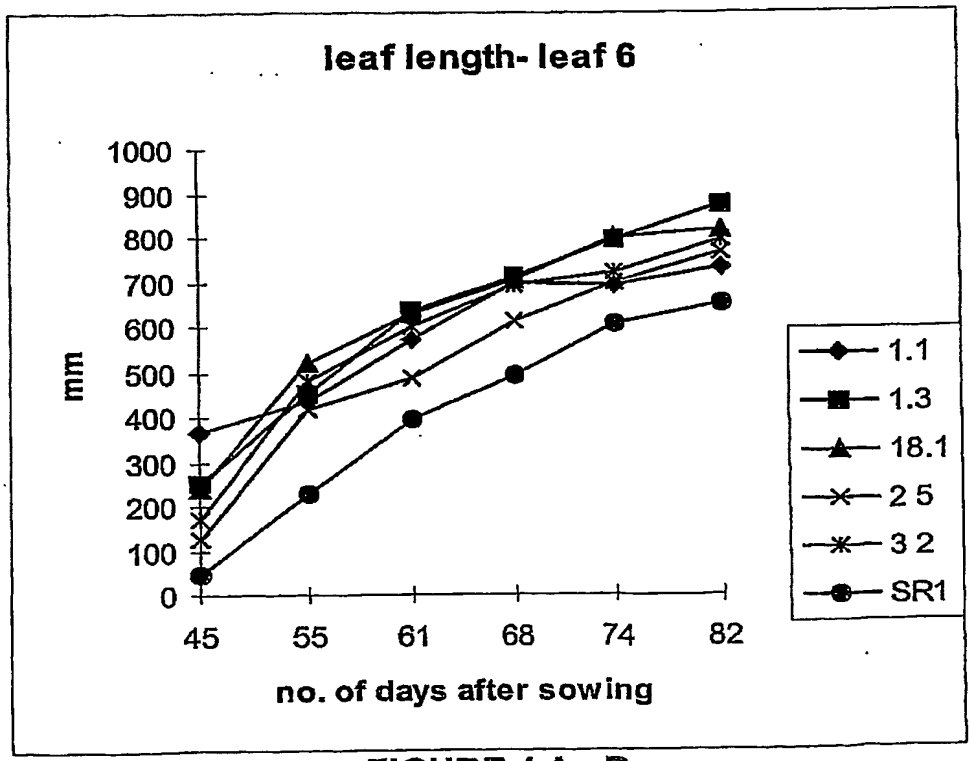
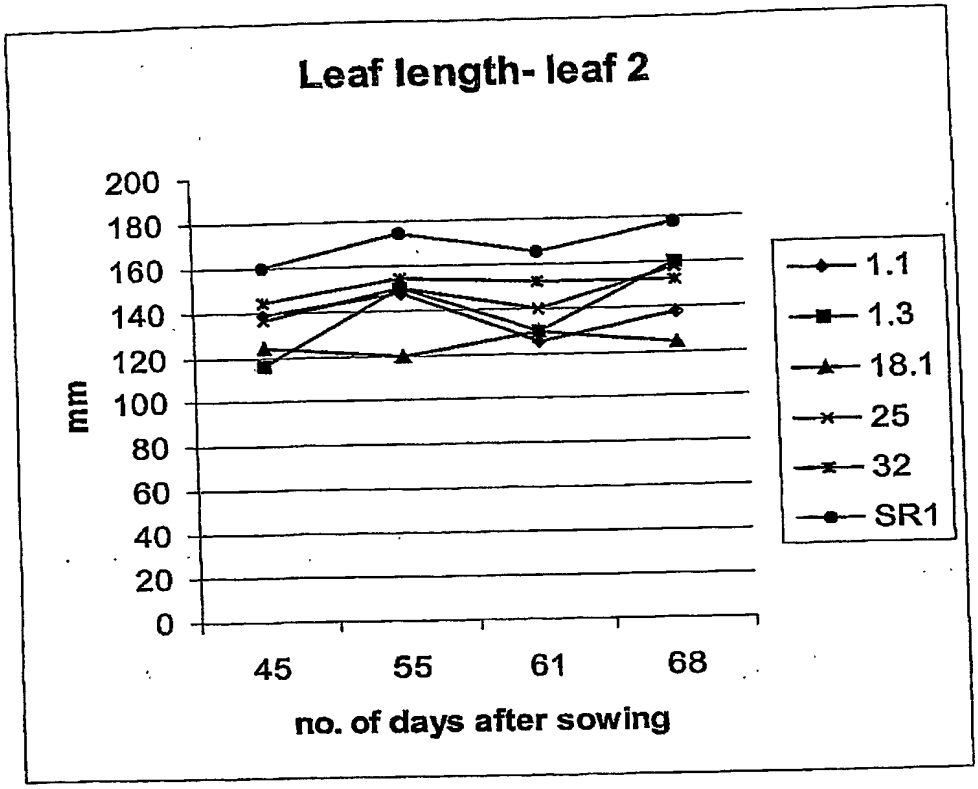


FIGURE 4 A - B

C



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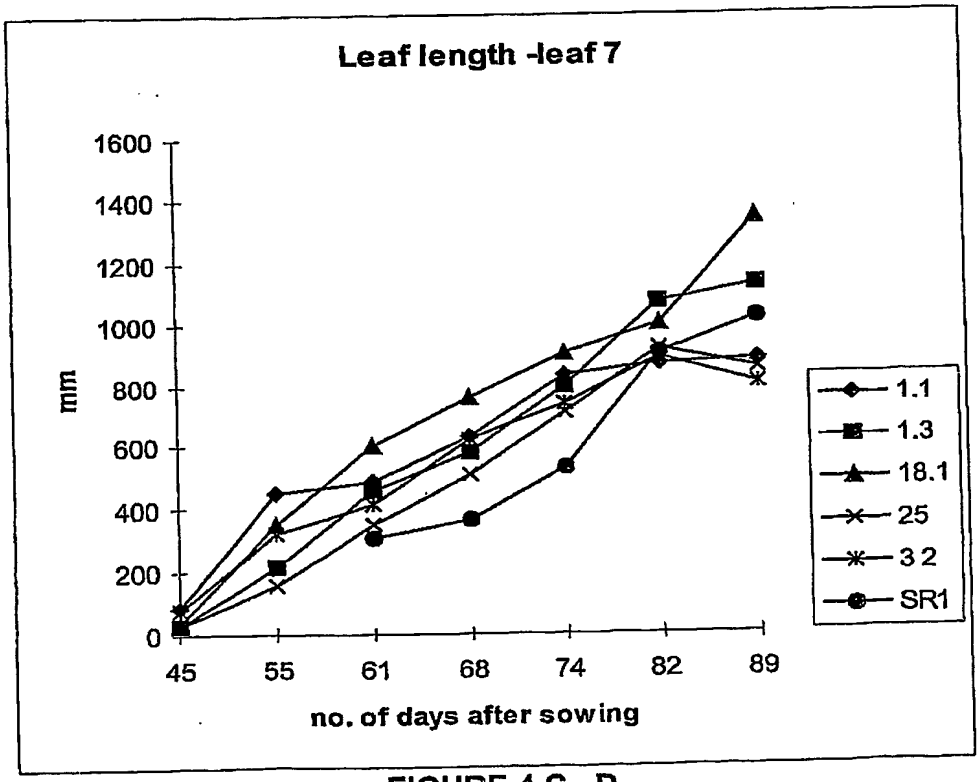
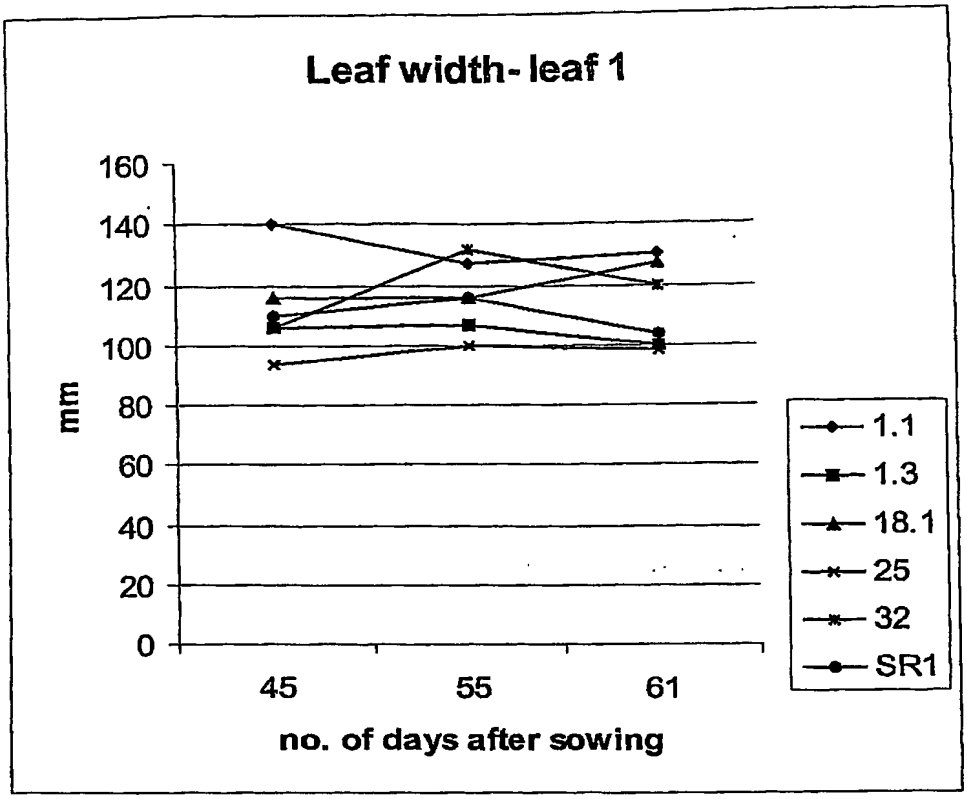


FIGURE 4 C - D

A



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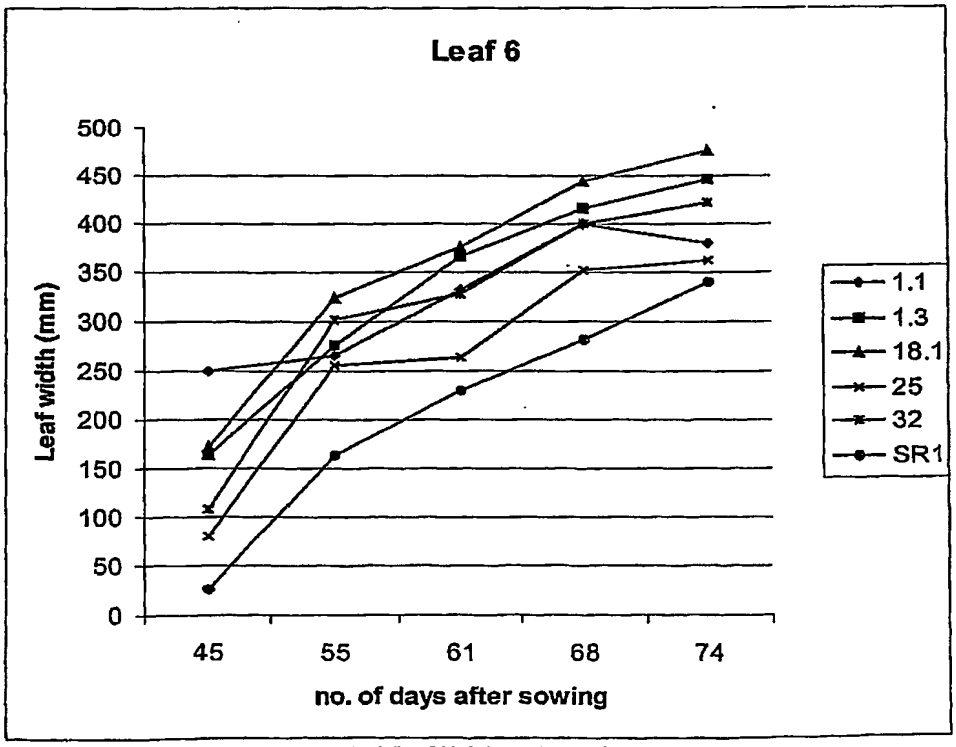
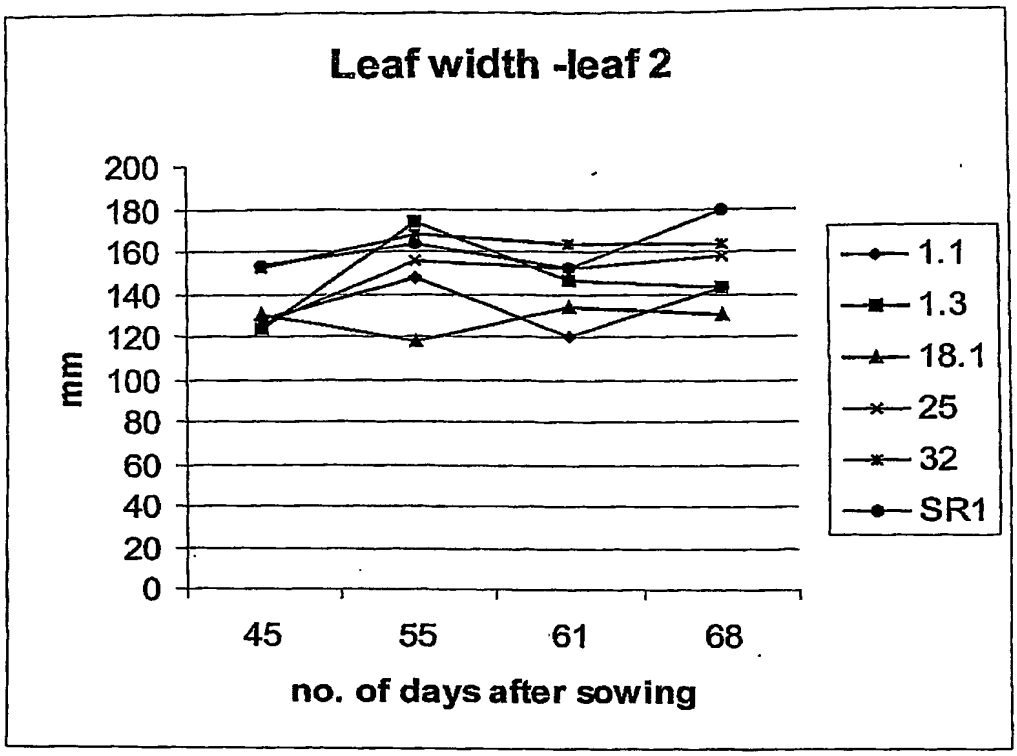


FIGURE 5 A - B

C



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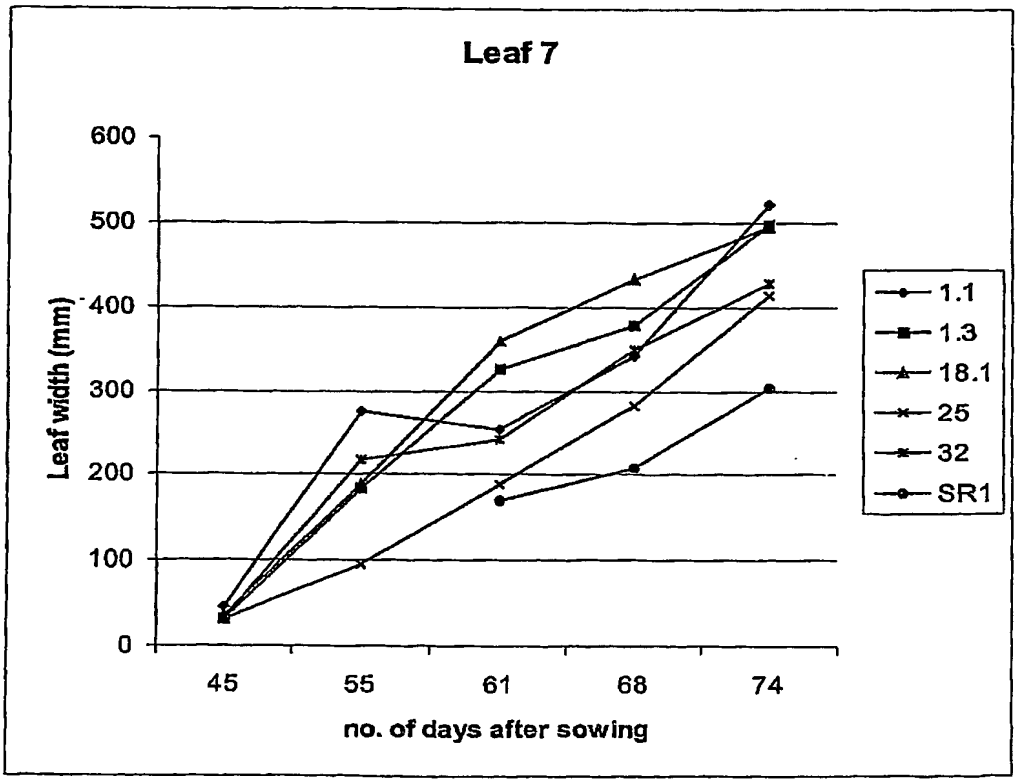

FIGURE 5 C - D

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AA

FIGURE 6

SEQ ID NO 2 protein Arabidopsis thaliana cdc27A1

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FIGURE 6 (CONTINUED)

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Universidade Federal do Rio de Janeiro

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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Coop eration Treaty.

For receiving Office use only

International Application No. PCT/EP 02 / 10265

International Filing Date (05. 09. 2002) 05 SEP 2002

Name of receiving Office and "PCT International Application": EUROPEAN PATENT OFFICE PCT INTERNATIONAL APPLICATION

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 57-cdc27-wo

Box No. I TITLE OF INVENTION

Plants having modified development and a method for making the same

Box No. II APPLICANT ☐ This person is also inventor

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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B-9052 Zwijnaarde
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Telephone No. +32 9 241 50 80

Facsimile No. +32 9 241 50 89

Teleprinter No.

Applicant's registration No. with the Office

State *(that is, country)* of nationality: BE

State *(that is, country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
CEP 21941 - 590 Rio de Janeiro, RJ
Brazil

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: BR

State *(that is, country)* of residence: BR

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☐ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

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Belgium

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Facsimile No. +32 9 241 50 89

Teleprinter No.

Agent's registration No. with the Office

☒ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

CONFIRMATION COPY

Continuation of Box No. III **FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)**

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

FERREIRA, Paulo Cavalcanti Gomes
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Leblon
CEP 22430-090 Rio de Janeiro, RJ
Brazil

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: BR

State *(that is, country)* of residence: BR

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HEMERLY, Adriana Silva
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CEP 22430-090 Rio de Janeiro, RJ
Brazil

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality: BR

State *(that is, country)* of residence: BR

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ROMBAUTS Stéphane
Nederpolder 23
B-9000 Gent
Belgium

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

Applicant's registration No. with the Office

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES *Mark the applicable check-boxes below; at least one must be marked.*

The following designations are hereby made under Rule 4.9(a):

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZM Zambia, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, BG Bulgaria, CH & LI Switzerland and Liechtenstein, CY Cyprus, CZ Czech Republic, DE Germany, DK Denmark, EE Estonia, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, SK Slovakia, TR Turkey, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GQ Equatorial Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AG Antigua and Barbuda
☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ BZ Belize
☒ CA Canada
☒ CH & LI Switzerland and Liechtenstein
☒ CN China
☒ CO Colombia
☒ CR Costa Rica
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ DM Dominica
☒ DZ Algeria
☒ EC Ecuador
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IN India
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakhstan
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ MZ Mozambique
☒ NO Norway
☒ NZ New Zealand
☒ OM Oman
☒ PH Philippines
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia
☒ SK Slovakia
☒ SL Sierra Leone
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TN Tunisia
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ TZ United Republic of Tanzania
☒ UA Ukraine
☒ UG Uganda
☒ US United States of America
☒ UZ Uzbekistan
☒ VN Viet Nam
☒ YU Yugoslavia
☒ ZA South Africa
☒ ZM Zambia
☒ ZW Zimbabwe

Check-boxes below reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ VC Saint Vincent and the Grenadines ☐ ☐
☒ SC Seychelles [AA] ☐ ☐

AA ex officio RO/EP

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM

The priority of the following earlier application(s) is hereby claimed:

Filing date of earlier application *(day/month/year)*	Number of earlier application	Where earlier application is:		
		national application: country or Member of WTO	regional application:* regional Office	international application: receiving Office
item (1)				
item (2)				
item (3)				
item (4)				
item (5)				

☐ Further priority claims are indicated in the Supplemental Box.

The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office)* identified above as:

☐ all items ☐ item (1) ☐ item (2) ☐ item (3) ☐ item (4) ☐ item (5) ☐ other, see Supplemental Box

** Where the earlier application is an ARIPO application, indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed (Rule 4.10(b)(ii)):* .....

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:

ISA / EP .....

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII DECLARATIONS

The following declarations are contained in Boxes Nos. VIII (i) to (v) *(mark the applicable check-boxes below and indicate in the right column the number of each type of declaration)*: Number of declarations

☐ Box No. VIII (i) Declaration as to the identity of the inventor :

☐ Box No. VIII (ii) Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent :

☐ Box No. VIII (iii) Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application :

☐ Box No. VIII (iv) Declaration of inventorship (only for the purposes of the designation of the United States of America) :

☐ Box No. VIII (v) Declaration as to non-prejudicial disclosures or exceptions to lack of novelty :

Box No. IX CHECK LIST; LANGUAGE OF FILING

This international application **contains:**

(a) the following number of **sheets in paper form:**

request (including declaration sheets)	:	5
description (excluding sequence listing part)	:	13
claims	:	2
abstract	:	1
drawings	:	8
Sub-total number of sheets	:	29
sequence listing part of description *(actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (b) below)*	:	6
Total number of sheets	:	35

(b) sequence listing part of description filed in **computer readable form**

(i) ☐ only (under Section 801(a)(i))

(ii) ☐ in addition to being filed in paper form (under Section 801(a)(ii))

Type and number of carriers (diskette, CD-ROM, CD-R or other) on which the sequence listing part is contained *(additional copies to be indicated under item 9(ii), in right column)*: .....

This international application is **accompanied by** the following item(s) *(mark the applicable check-boxes below and indicate in right column the number of each item)*:

		Number of items
1. ☐	fee calculation sheet :	
2. ☐	original separate power of attorney :	
3. ☐	original general power of attorney :	
4. ☐	copy of general power of attorney; reference number, if any: ..... :	
5. ☐	statement explaining lack of signature :	
6. ☐	priority document(s) identified in Box No. VI as item(s): ..... :	
7. ☐	translation of international application into *(language)*: ..... :	
8. ☐	separate indications concerning deposited microorganism or other biological material :	
9. ☐	sequence listing in computer readable form (indicate also type and number of carriers (diskette, CD-ROM, CD-R or other))	
	(i) ☐ copy submitted for the purposes of international search under Rule 13*ter* only (and not as part of the international application) :	
	(ii) ☐ *(only where check-box (b)(i) or (b)(ii) is marked in left column)* additional copies including, where applicable, the copy for the purposes of international search under Rule 13*ter* :	
	(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing part mentioned in left column :	
10. ☒	other *(specify)*: Letter. ..... :	1

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Meeta MISTRY
Intellectual Property Group Leader
CropDesign N. V.

M. Mistry

For receiving Office use only

1. Date of actual receipt of the purported international application: 05 SEP 2002 (05. 09. 2002)

2. Drawings: ☑ received: ☐ not received:

3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:

4. Date of timely receipt of the required corrections under PCT Article 11(2):

5. International Searching Authority (if two or more are competent): ISA /

6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

Plants having modified development and a method for making the same

Background

The regulation of the cell cycle in plants is poorly understood. Most of the knowledge regarding the regulation of DNA replication, also known as the S-phase of the cell cycle regulation originates from experimental data obtained in yeast and mammalian cells. However, the importance to understand the cell cycle regulation in plant cells has become increasingly important in agriculture, e.g. to ensure survivability of plants in unfavourable growth conditions, or to obtain resistance or tolerance against pathogens, and/or to improve economically important agronomic characteristics such as yield.

Detailed description

The inventors of the present application previously described the isolation of the cdc27A gene (SEQ ID NO 1), encoding a protein as in SEQ ID NO 2) and a splice variant thereof (WO 01/02430), as a gene involved in DNA synthesis.

Surprisingly it has now being found that introduction of a cdcd27A in Tobacco plants, gives rise to plants having altered developmental characteristics. In particular it was unexpected that the transgenic plants showed increased rate of development. This beneficial effect was in balance with the overall architecture and shape of the plant and the acceleration of the plant development resulted in healthy adult plants that reach the flowering stage earlier than control plants. Additionally, the adult plants appeared to grow larger. The effects of the introduction of a transgene becomes progressively more pronounced in the later stages of development of the plant. It was further surprising to find the same plants having an increased of the number of flowers and/or increased number of seeds.

These technical features are particularly favourable when commercially producing plants, because these technical features contribute to increase yield.

It has now being found that introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof gives rise to Plants having altered development.

Therefore according to the present invention there is provided a method for altering plant development, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof.

The effects of introducing the expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof can be any one or more of the following: enhanced transcript level, enhanced protein level, enhanced expression

of the encoded gene or decrease of expression of the native gene or enhanced activity of the encoded protein.

It has now being shown that by introducing a cdcd27a gene into a plant, it is possible to alter plant development and more particularly, alter one or more of the following phenotypic characteristics: faster development, early flowering, more flowers, more seeds, bigger leaves and increased height.

Accordingly a particular embodiment of the invention relates to a method as described above wherein said altering plant development comprises increasing the rate of development of a plant or plant part relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altering plant development comprises altered flowering characteristics relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altering flowering characteristics comprises altering the period of time to reach flowering relative to corresponding wild-type plants.

In a preferred embodiment the invention relates to a method as described above, wherein said altered period of time to reach flowering comprises early flowering relative to corresponding wild-type plants.

Plant development also includes organ formation. Accordingly, altering plant development includes altering the number of organs for example the number of flowers and/ or the number of seeds.

Accordingly, a preferred embodiment the present invention relates to a method as described above wherein said altering flowering characteristics comprises increasing the number of flowers relative to corresponding wild-type plants.

Accordingly, a preferred embodiment the present invention relates to a method as described above, wherein said altering plant development comprises increasing the number of seeds relative to corresponding wild-type plants.

Another aspect of the invention is the use of a nucleic acid encoding a cdcd27a protein. More particularly, the inventors cloned the cdcd27A encoding gene in an expression vector that is expressible in plants.

Accordingly, the invention also embodies a method as described above, wherein said plant-expressible vector comprises:

a) A nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof;

b) A regulatory sequence capable of driving expression of said nucleic acid sequence of a); and

c) Optionally, a terminator sequence.

According to a preferred embodiment of the present invention, a nucleic acid sequence encoding a cdc27A protein is a nucleic acid sequence encoding the cdcd27A1 protein from *Arabidopsis thaliana*

Further preferable, the cdc27A protein is cdc27A1 as depicted in SEQ ID NO 2

Alternatively, said cdcd27A1 protein is encoded by SEQ ID NO 1.

The present invention further relates to plants obtained by the methods of the present invention and showing one or more of the following specific phenotypes: faster development, early flowering, more flowers, more seeds, bigger leaves and increased height.

Another aspect of the invention is the transgenic plant that is obtained by the present invention. Accordingly, another embodiment of the present invention is a transgenic plant obtained by any method as described above, said plant having altered plant development.

Also, another embodiment of the invention relates to the ancestors or progeny of a plant as described above, said ancestor or progeny having altered plant development relative to corresponding wild type plants

Definitions

"altering development" as described herein encompasses increased (faster or accelerated) rate of development or a decreased rate of development or induced development at any one or more developmental stages of the life cycle of a plant.

Increased development encompasses an increase in the rate of progression through one or more, or part of one or more, of the different stages of the life cycle of the plant. The life cycle of a plant encompasses any stage from a dormant seed to a senescing plant and any stage in between. Further more accelerated development also encompasses stages leading up to seed formation.

One can envisage a situation where a plant can grow faster, but where the plant does not reach a certain developmental stage.

According to the present invention, the term "altered flowering characteristics" encompasses one or more of the following: altered period of time to reach flowering, altered number of flowers, altered length flowering period, each relative to corresponding wild type plants

According to the invention, the "gene" or the "polypeptide" may be the wild type, i.e. the native or endogenic nucleic acid or polypeptide which expression is modified. Alternatively, the gene may be a heterologous nucleic acid derived from the same or another species and be introduced as a transgene for example by transformation. This transgene may be substantially modified from its native form in composition and/or genomic environment through deliberate

human manipulation. Also expression of the native genes can be modified introduction in the plant of regulatory sequences that alter the expression of the native gene.

One way of modifying the expression of cdcd27A according to the invention relates to a method comprising the stable integration into the genome of a plant or in specific plant cells or tissues of said plant of an expressible gene encoding a plant cdc27, a homologue or a derivative thereof or an enzymatically active fragment thereof.

The term "overexpression" should be understood as "ectopic expression". "Ectopic expression" or "ectopic overexpression" of a gene or a protein refers to expression patterns and/or expression levels of said gene or protein normally not occurring under natural conditions. Ectopic expression can be achieved in a number of ways including operably linking of a coding sequence encoding said protein to an isolated homologous or heterologous promoter in order to create a chimeric gene and/or operably linking said coding sequence to its own isolated promoter (i.e. the unisolated promoter naturally driving expression of said protein) in order to create a recombinant gene duplication or gene multiplication effect.

According to at least one preferred embodiment of the invention, enhanced or increased expression of said nucleic acid is envisaged. Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and are for example overexpression driven by a strong promoter, the use of transcription enhancers or translation enhancers. Examples of decreasing expression are also well documented in the art and are for example: downregulation of expression by anti-sense techniques, gene silencing etc.

The term "overexpressing" in the present invention also means modifying the activity of the gene product, more particularly in the present invention the cdc27A protein in the host cell. This can be achieved for example by respectively inhibiting or stimulating the control elements that drive the expression of the native gene or of the transgene. Also modifying the activity of the gene product, the polypeptide, can furthermore be achieved by administering or exposing cells, tissues, organs or organisms to, an interacting protein or an inhibitor or activator of said gene product. In the context of the present invention, such inhibitors or activators can also effect their activity against the cdcd27A protein in order to obtain the effects as claimed in the present invention.

Also modifying, increasing, the activity of the gene product, the polypeptide, can furthermore be achieved by administering or exposing cells, tissues, organs or organisms to, a preparation of said gene product, so that it can exert its functions in said exposed cells or tissues. In the context of the present invention, the cells are exposed to protein samples of cdcd27A protein.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant cell" also encompasses suspension cultures, embryos,

meristematic regions, callus tissue, leaves, seeds, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

Advantageously, the method according to the present invention is applicable to algae, fungi, angiosperms and gymnosperms, both monocotyledonous and dicotyledonous plants. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp.,Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp.,Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp.Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays*, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot,

cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, trees and algae amongst others. According to a preferred feature of the present invention, the plant is a monocotyledonous plant, further preferably a crop plant such as rice, maize, wheat, barley, soybean, sunflower, canola, alfalfa, millet, barley, rapeseed and cotton.

A "vector" as used herein refers to a nucleic acid used for transfection or transformation of a host cell and into which a nucleic acid sequence can be inserted. Expression vectors allow transcription and/or translation of a nucleic acid inserted therein. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. The term "expressible" or "plant-expressible" relates to the presence of control sequences which promote adequate expression of genes and/or proper translation of said sequences into a protein.

The terms "gene(s)", "polynucleotide(s)", "nucleic acid(s)", "nucleotide sequence(s)", "nucleic acid sequences" or "nucleic acid molecules", are used interchangeably herein and encompass a polymeric form of a deoxyribonucleotide or a ribonucleotide polymer of any length, either double- or single-stranded, or analogues thereof, that have the essential characteristic of a natural ribonucleotide in that they can hybridise to nucleic acids in a manner similar to naturally occurring polynucleotides.

A great variety of modifications, which are known in the art, can be made to DNA and RNA and can serve many useful purposes. Such modifications include methylation, 'caps' and substitution of one or more of the naturally occurring nucleotides with an analogue.

"Sense strand" refers to a DNA strand that is homologous to an mRNA transcript thereof; "anti-sense strand" refers to an inverted sequence which is complementary to the "sense strand".

"Encoding" or "encodes", with respect to a specified nucleotide sequence, refers to the information for translation into a specified protein. A nucleic acid encoding a protein may contain non-translated sequences such as 5' and 3' untranslated regions (5' and 3' UTR) and introns or it may lack intron sequences, such as in cDNAs.

An "open reading frame" or "(ORF)" is defined as a nucleotide sequence that encodes a polypeptide. The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the 'universal' genetic code but variants of this universal code exist (see for example Proc. Natl. Acad. Sci. U.S.A 82: 2306-2309 (1985)). The boundaries of the coding sequence are determined by a translation start codon at the 5'-end and a translation stop codon at the 3'-terminus. Because of the degeneracy of the genetic code, a large number of nucleic acids can encode any given protein. As such, substantially divergent nucleic acid sequences can be designed to effect expression of essentially the same protein in different hosts. Conversely, genes and coding sequences essentially encoding the same protein isolated from different sources can consist of substantially different nucleic acid sequences.

Methods for the search and identification of homologues of CDC27A, would be well within the realm of a person skilled in the art. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information. The cdc27A homologue from *Arabidopsis* identified above was identified using BLAST default parameters.

"Homologues" encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or additions relative to the protein in question and having similar biological and functional activity as an unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company). The homologues useful in the method according to the invention most preferably have a degree of sequence identity or similarity (functional identity) to, for example, the unmodified CDC27A protein, wherein said degree of sequence identity or similarity is typically at least 50%, more typically at least 60%, preferably at least 70%, further preferably at least 80%, most preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% 99% sequence identity or similarity to an unmodified CDC27A.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "parologous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins according to the invention.

"Substitutional variants" of a protein of the invention are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions.

"Insertional variants" of a protein of the invention are those in which one or more amino acid residues are introduced into a predetermined site in said protein. Insertions can comprise

amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, $(histidine)_6$-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein of the invention are characterised by the removal of one or more amino acids from the protein. Amino acid variants of a protein of the invention may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

"Derivatives" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring cdc27A protein.

"Active fragments" or "functional fragment" of CDC27A encompasses at least five contiguous amino acid residues of a CDC27A protein, which residues retain similar biological and/or functional activity to the naturally occurring CDC27A.

Also allelic variants and splice variants of the cdcd27A protein can be used for the methods of the present invention;

The expression "introducing" a plant expressible vector relates any transformation technique.

The recombinant DNA constructs for use in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The recombinant gene constructs may be inserted into vectors, which may be

commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.

Optionally, the gene of interest may be associated with a selectable marker gene. Such a marker gene encodes a trait or a phenotype which allows the selection of a plant or plant cell containing the marker. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance. Cells containing the recombinant DNA will thus be able to survive in the presence of antibiotic or herbicide concentrations that kill untransformed cells. Examples of selectable marker genes include the *bar* gene which provides resistance to the herbicide Basta; the npt gene which confers resistance to the antibiotic kanamycin; the hpt gene which confers hygromycin resistance. Visual markers, such as the Green Fluorescent Protein (GFP) may also be used as selectable markers. An entire plant may be generated from a single transformed plant cell through cell culturing techniques known to those skilled in the art.

The gene of interest is introduced into a plant by transformation. The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid, or alternatively, may be integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. Transformation methods include the use o agrobacterium, liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Preferable a preferred method is leaf disk transformation. Alternative, methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. *et al.*, 1882, Nature 296, 72-74; Negrutiu I. *et al.*, June 1987, Plant Mol. Biol. 8, 363-373); electroporation of protoplasts (Shillito R.D. *et al.*, 1985 Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A. *et al.*, 1986, Mol. Gen Genet 202, 179-185); DNA or RNA-coated particle bombardment (Klein T.M. *et al.*, 1987, Nature 327, 70) infection with (non-integrative) viruses and the like. A preferred method according to the present invention comprises the protocol according to Hiei *et al.*1994.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced

DNA may be undertaken using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The present invention also provides plants obtained by the method according to the present invention.

According to the present invention, plants are transformed with a vector comprising the sequence of interest (i.e., a nucleic acid sequence comprising CDC27A), wherein said gene is operably linked to a promoter, preferably to a constitutive.

The terms "regulatory element", "control sequence" "promoter" are all used herein interchangeably and taken in a broadest context refer to regulatory nucleic acid sequences capable of effecting expression of the sequences to which they are ligated. Encompassed by the aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a –35 box sequence and/or –10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. The terms "control sequence", "regulatory sequence", "regulatory element" and "promoter" are used interchangeably herein. The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Examples

The present invention will now be described with reference to the following examples, which are by way of illustration alone.

Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York; or in Volumes 1 and 2 of Ausubel *et al.* (1984), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

In a particular example of the present invention, said cdc27A gene is isolated and cloned in a plant expressable vector carrying the CaMV35S promoter.

In a particular example of the present invention, the CaMV35S::cdcd27A construct is subsequently transformed and stably integrated in the genome of Tobacco.

In a particular example of the present invention the cdcd27A gene is a dcd27A gene of *Arabidopsis thaliana* or is a gene as presented in SEQ ID NO 1, encoding the cdc27A protein as presented in SEQ ID NO 2. Alternatively other homologues, allelic variants and splice variants can be used for the methods of the present invention.

Example of cloning cdcd27A1:

To express constitutively the Arabidopsis CDC27a cDNA in transgenic plants, PCR mutagenesis has been carried out to introduce restriction sites in the full-length cDNA. Oligonucleotides containing NcoI and BamHI restriction sites were used in a PCR reaction, the resulting fragment was restricted with the 2 enzymes (NcoI and BamHI). The CDC27a reading frame was ligated in the PH35S (Hemerly et al. EMBO J. 14, 3925-3936), in the same sites, in front of the 35S promoter, and with the NOS terminator. The resulting plasmid was digested with EcoRI, filled in with Klenow enzyme, and then cut with SalI to release a fragment contaning the 35S promoter, the CDC27 reading frame, and the NOS terminator. This fragment was cloned in the PGSV4 plasmid in the SalI and ScaI sites. The resulting plasmid was introduced in Agrobacterium tumefasciens C58.

Tobacco plants were transformed with the resulting Agrobacterium strain. Seeds of the tobacco T1 plants were germinated in medium containing kanamycin to determine the number of copies of the transgene. Plants with a 3 to 1 relation of kanamycin resistant to susceptible seedlings were chosen to produce seeds in order to obtain homozigous plants.

Example of transformation of tobacco plants

For introduction of the gene encoding the cdcd27A protein into tobacco plants, the leaf disk method was used . This method is previously described in Horsch,R.B. et al. (1985) A simple and general method for transferring genes into plants Science 227 1229 – 1231.

Description of the Figures

The present invention will now be described with reference to the following figures in which:

Figure 1 is a schematic representation of the construct used for transforming the plants of the present invention.

Figure 2 illustrates that newly developed leaves develop faster in cdcd27A transgenic plants compared to control plants (SR1). Number 1 to 6 correspond to the leaves as they grow on the stem of the plant, meaning that leaf 1 is the leaf developed in the juvenile plant and leaf

6 being the most recently developed leaf, i.e. a leaf developed when the same plant is in a more mature stage. The data illustrate that the effects of introduction of a cdc27A gene into a plant on plant development becomes progressively more pronounced as the plant matures.
The lower line designated 35S13.3/1, shows the leaves of a transgenic plant transformed with 35S::cdc27A1 and the upper line designated SR1 shows the leaves of a non transgenic control plant. These pictures illustrate that the transgenic leaves are bigger and appear earlier during the plant life cycle compared to the control plant. The results illustrate that transgenic plants have an accelerated rate of development. The results further illustrate that, at the same developmental stage, transgenic leaves are bigger relative to the leaves of control plants, indicating that the transgenic pants have increased growth.

Figure 3 illustrates transgenic plants transformed with 35S::cdc27A (positions 1 to 4) and a non transgenic control plant (position 5). The transgenic plants as seen in the illustration are taller, thereby indicating faster development. Additionally the transgenic plants flower earlier than the control plant as also illustrated in the figure. The picture shows that transgenic plants that develop faster reach an adult stage faster than the control plant without growth arrest or any apparent deformation or any apparent aberrant development during their life cycle.

Figure 4 (A to C) is a graphical illustration indicating that more recent leaves (leaves of the more mature plant, B and D) of the transgenic 35S::cdc27A1 plants develop quicker than leaves of a more juvenile transgenic plant (A and C). This is illustrated by the graphs indicating the length of the leaves number 1, 2, 6 and 7. This numbering corresponds to the leaf numbering of Figure 2. It is illustrated that the leaf length of the newly developed leaves 6 and 7 of the transgenic plant lines (1.1, 1.3, 18.1, 25, 3.2) is higher than that of control line SR1.

Figure 5 is a graphical representation illustrating that more recent leaves (i.e. of a more mature plant, B and D) of the transgenic 35S::cdc27A1 plants develop quicker than leaves of a more juvenile plant (A and C). This is illustrated by the graphs indicating the width of the leaves number 1, 2, 6 and 7. This numbering corresponds to the leaf numbering of Figure 2. It is illustrated that the leaf width of the newly developed leaves 6 and 7 of the transgenic plant lines (1.1, 1.3, 18.1, 25, 3.2) is higher than of the control line SR1.

Figure 6 is the nucleic acid sequence and protein sequence of the *Arabidopsis thaliana* cdcd27A protein.

Table 1 : Transgenic plants transformed with cdc27A flower earlier that control plants (see column flowering time). Also, transgenic plants are taller when they have reached the flowering stage compared to the non transgenic control plant (see column plant height at flowering time). From these data it is concluded that the transgenic plants show faster development, that they grow taller and that they flower early.

Table 1

Line	genotype	flowering time, mean after sowing (in days)*	Plant height mean at flowering time (cm)*	leaves number at flowering time*	Leave length/with ratio
1.1	homozygous	126,5 +- 11,13	63,8 +- 11,77	19,25 +- 1,98	1,87 +- 0,327 ***
1.3	homozygous	123,3 +- 16,66	6,3 +- 24,12	17,6 +- 3,75	1,76 +- 0,36 ***
18.8	hemizygous	124,8 +- 7,17	59,9 +- 16,70	18,2 +- 1,61	1,69 +- 0,28 ***
25 **	homozygous	138,5 +- 20,30	37,87 +- 19,98	17 +- 3,65	1,95 +- 0,27 ***
32	homozygous	127,2 +- 7,79	41,1 +- 11,11	16,8 +- 1,28	1,71 +- 0,28 ***
SR1	no transgene	147,6 +- 16,30	29 +- 4,6	17,8 +- 1,35	1,78 +- 0,13 ***

* 95%

** three of five plants

*** Mmean ± SD calculated from leave 6 of five plants with 74 days ($p < 0,05$)

Table 2: Transgenic plants transformed with 35S::cdcd27A1 have more flowers. Measurements involved five plants of each transgenic line and measurements of the control plants involved in the control line only two SR1 plants. These data illustrate that the introduction of cdcd27A in plant has an influence on plant development and more a particularly on organ formation, such as number of flowers. Furthermore it is envisaged that by using the methods of the present invention also the number of seeds is increased.

Line	1.1	1.3	18.1	25	32	SR1 *
	23,25	31,33	21,2	14	18,2	12,5

Claims

1. Method for altering plant development, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof.

2. Method according to claim 1 wherein said altering plant development comprises increasing the rate of development of a plant or plant part relative to corresponding wild-type plants.

3. Method according to claims 1 or 2, wherein said altering plant development comprises altered flowering characteristics relative to corresponding wild-type plants.

4. Method according to claims 3, wherein said altering flowering characteristics comprises altering the period of time to reach flowering relative to corresponding wild-type plants.

5. Method according to claims 4, wherein said altered period of time to reach flowering comprises early flowering relative to corresponding wild-type plants.

6. Method according to any of claims 3 to 5 wherein said altering flowering characteristics comprises increasing the number of flowers relative to corresponding wild-type plants.

7. Method according to any of claims 1 to 6, wherein said altering plant development comprises increasing the number of seeds relative to corresponding wild-type plants.

8. A method according to any of claims 1 to 7, wherein said plant-expressible vector comprises:

    a) A nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof;
    b) A regulatory sequence capable of driving expression of said nucleic acid sequence of a); and
    c) Optionally, a terminator sequence.

9. A method according to any of claims 1 to 8, wherein said cdc27A protein is cdc27A1 from *Arabidopsis thaliana*

10. A method according to any of claim 1 to 9, wherein said cdc27A protein is cdc27A1 as depicted in SEQ ID NO 2

11. Transgenic plant obtained by the method according to any of claims 1 to 10, said plant having altered plant development.

12. Ancestors or progeny of a plant according to claim 11, said ancestor or progeny having altered plant development relative to corresponding wild type plants

Abstract

Plants having modified development and a method for making the same

The present invention relates to a method for altering plant development, in particular altered flowering characteristics, comprising introducing into a plant, plant part or plant cell, a plant-expressible vector comprising a nucleic acid sequence encoding a cdc27A protein or a functional fragment, homologue or derivative thereof. Plants generated according to the invention show modified development and flowering characteristics

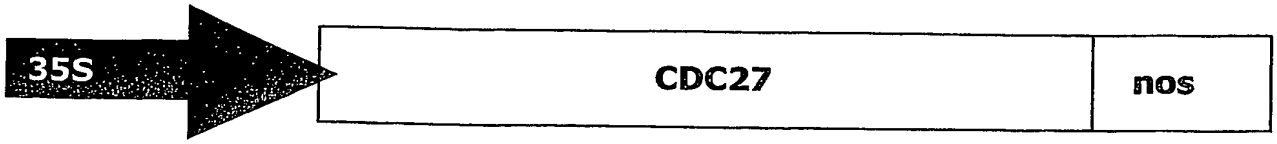


FIGURE 1

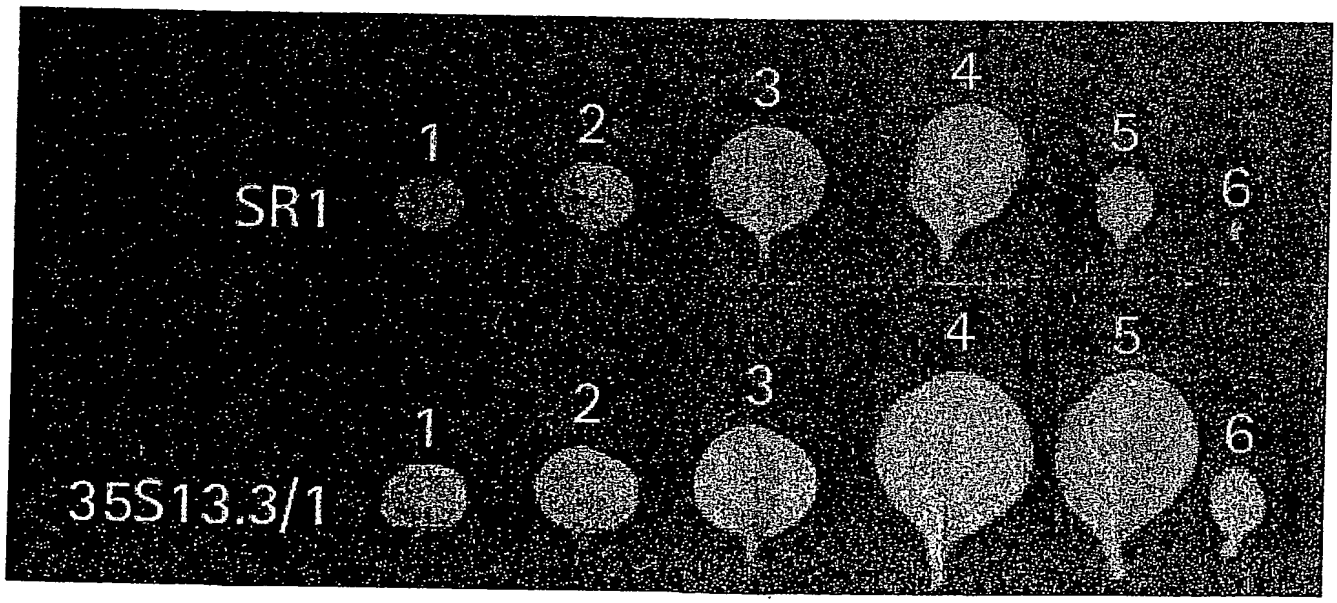
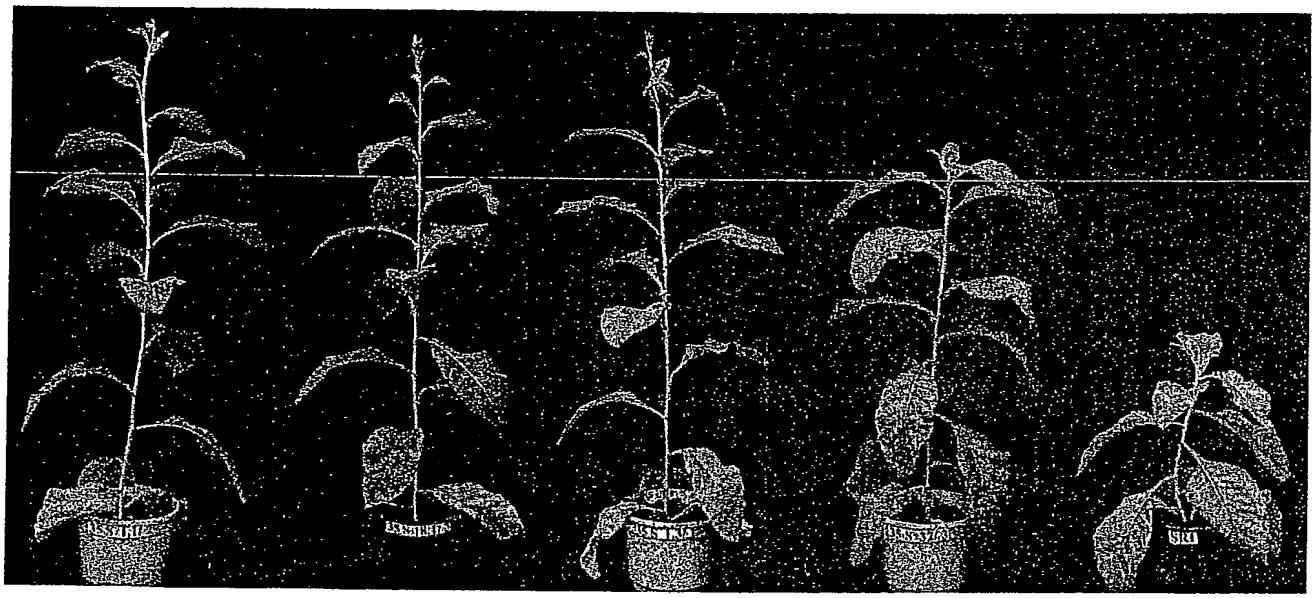


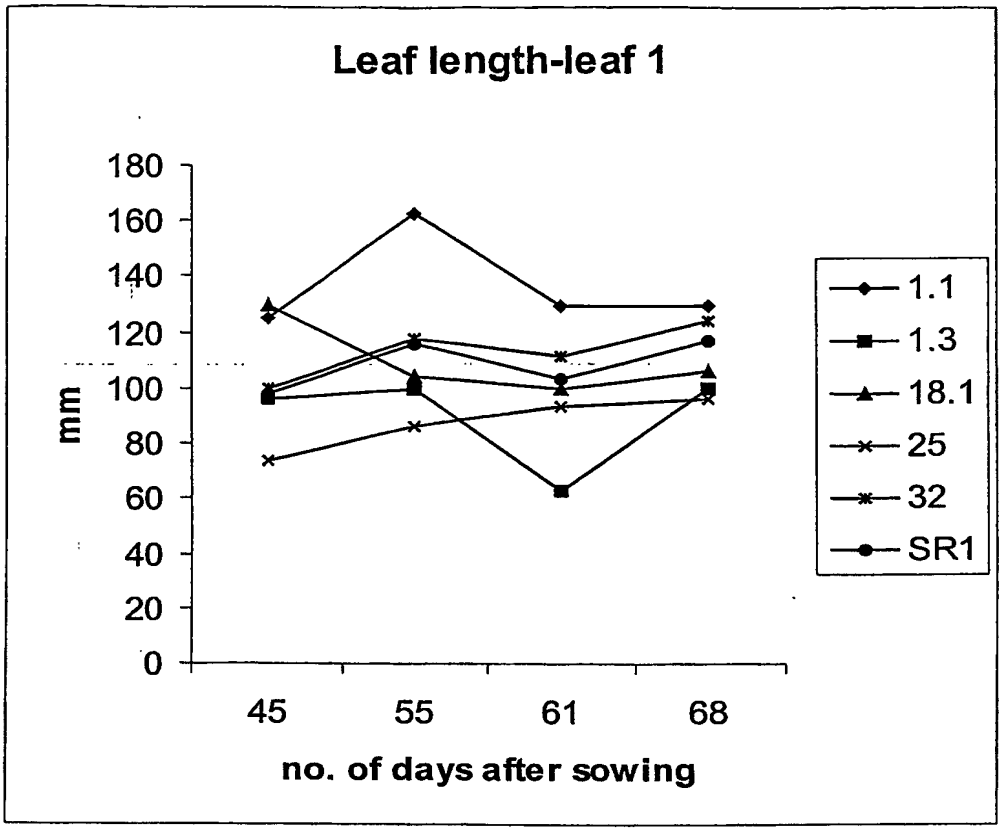
FIGURE 2



1 2 3 4 5

FIGURE 3

A



B

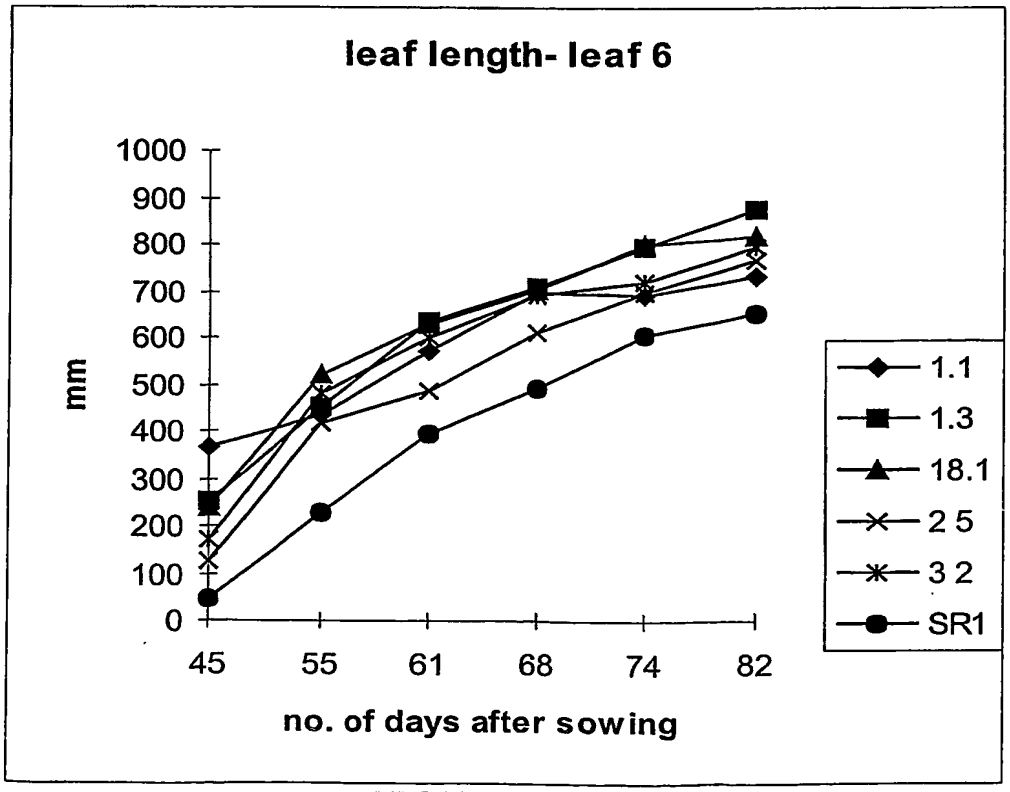
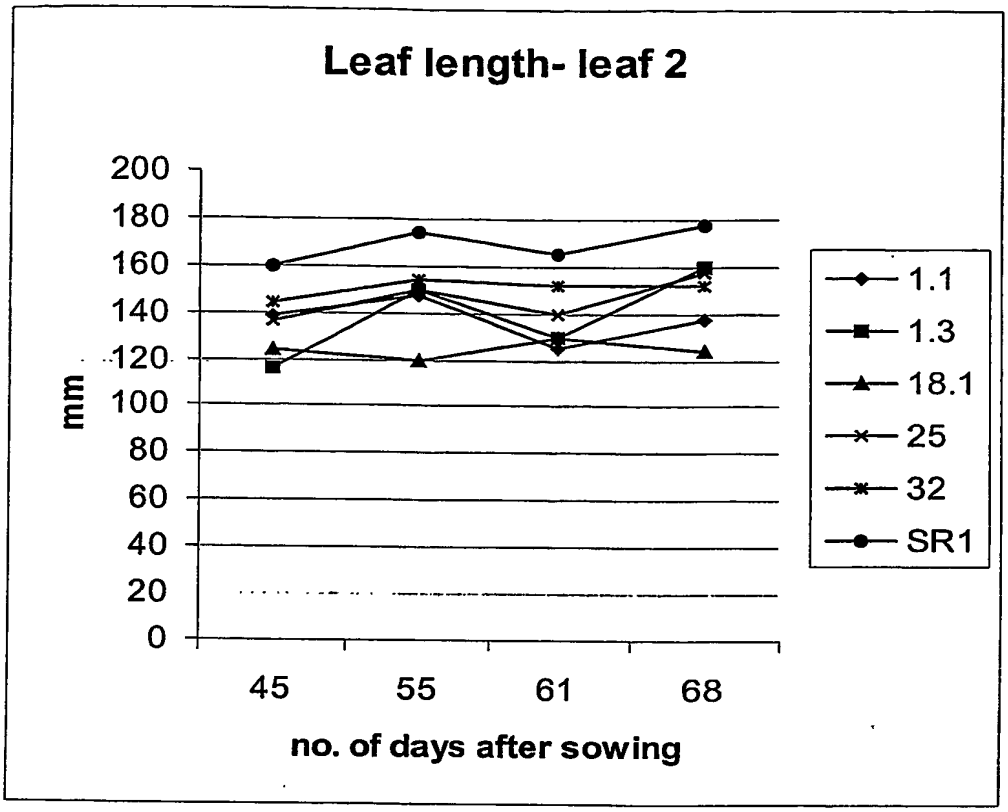


FIGURE 4 A - B

C



D

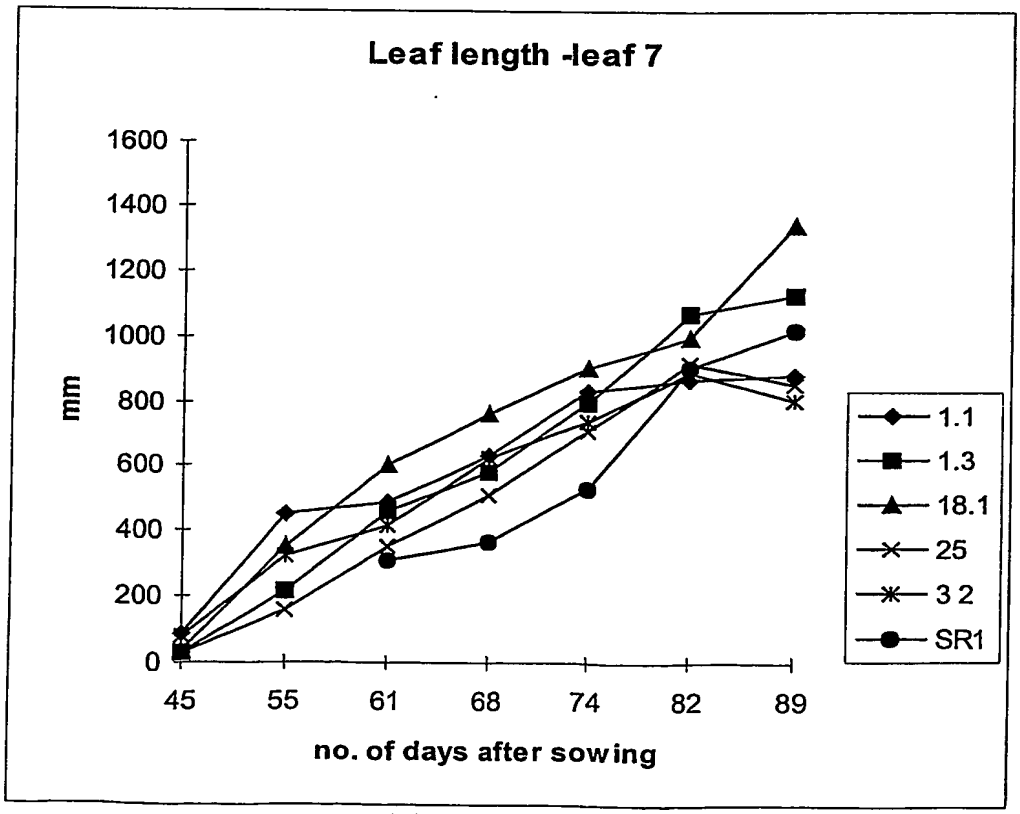
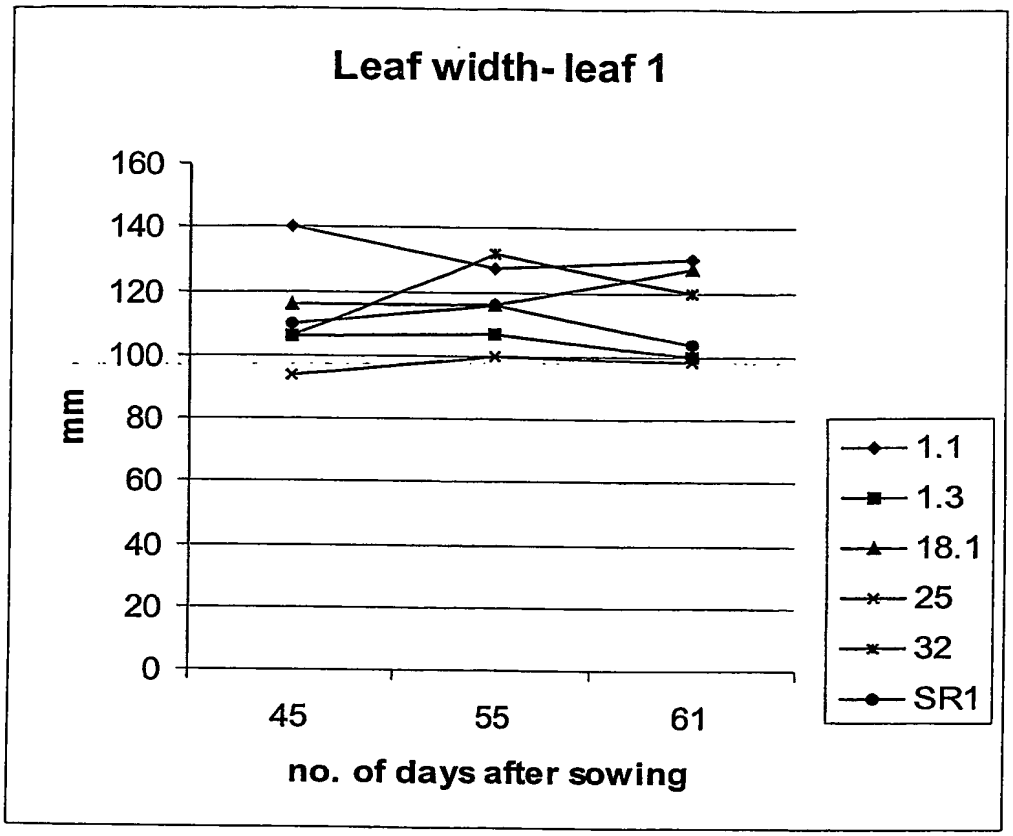


FIGURE 4 C - D

A



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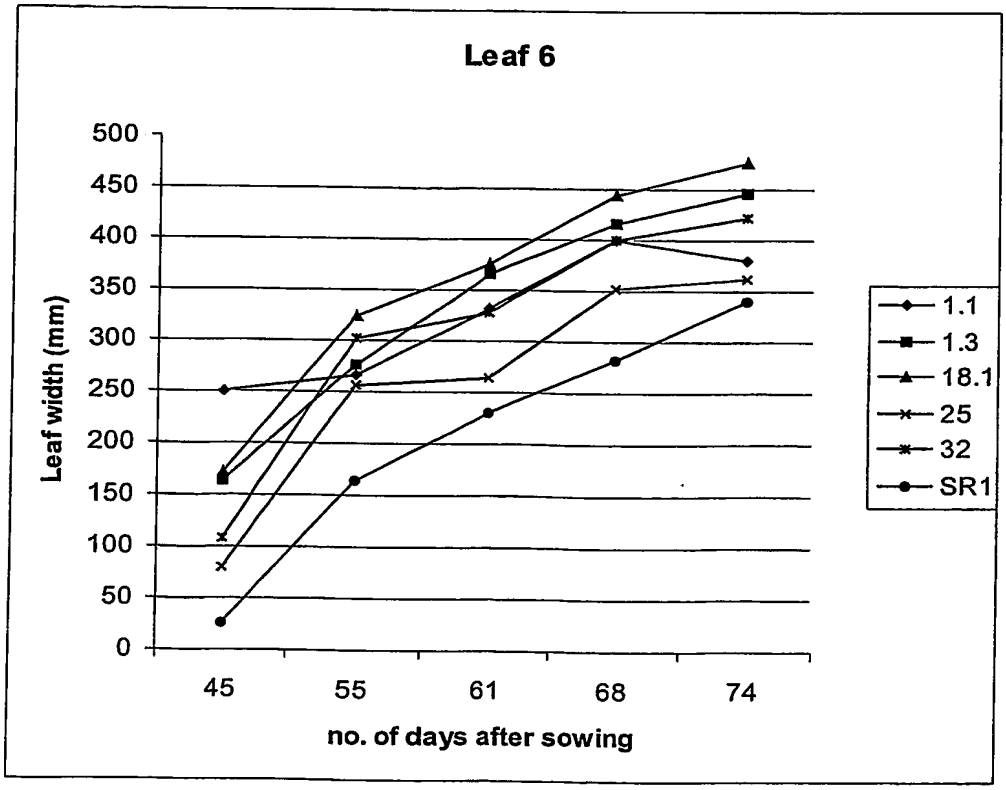
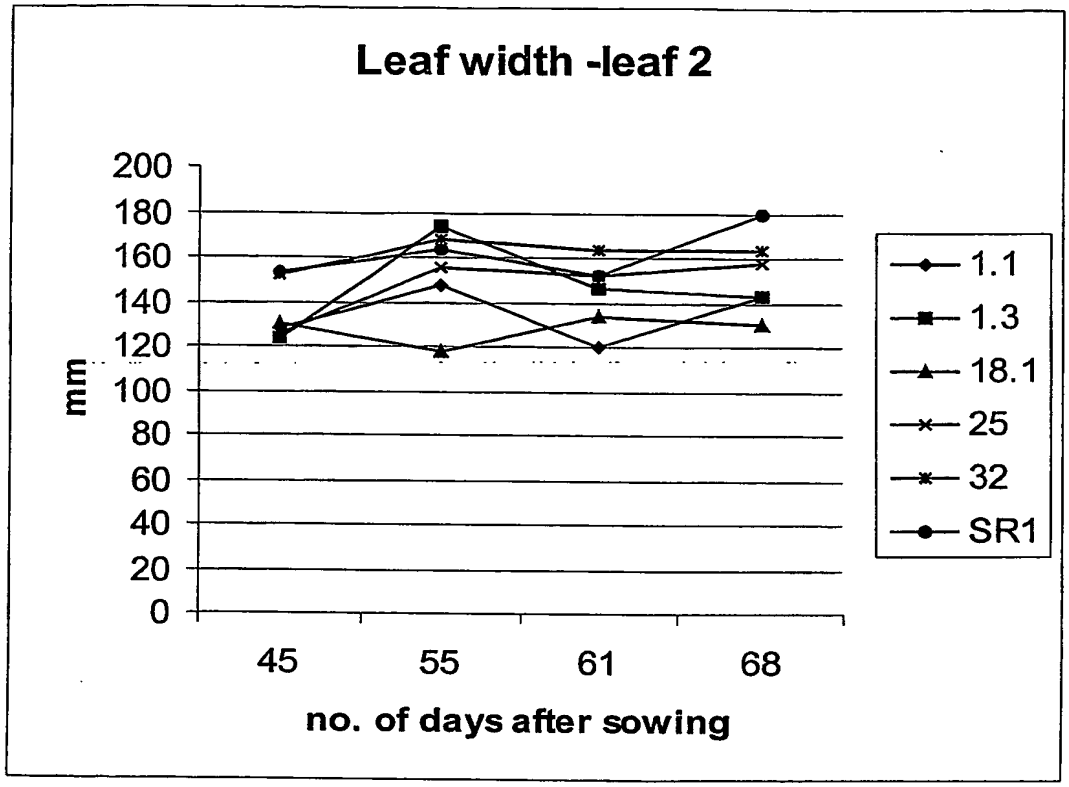


FIGURE 5 A - B

C



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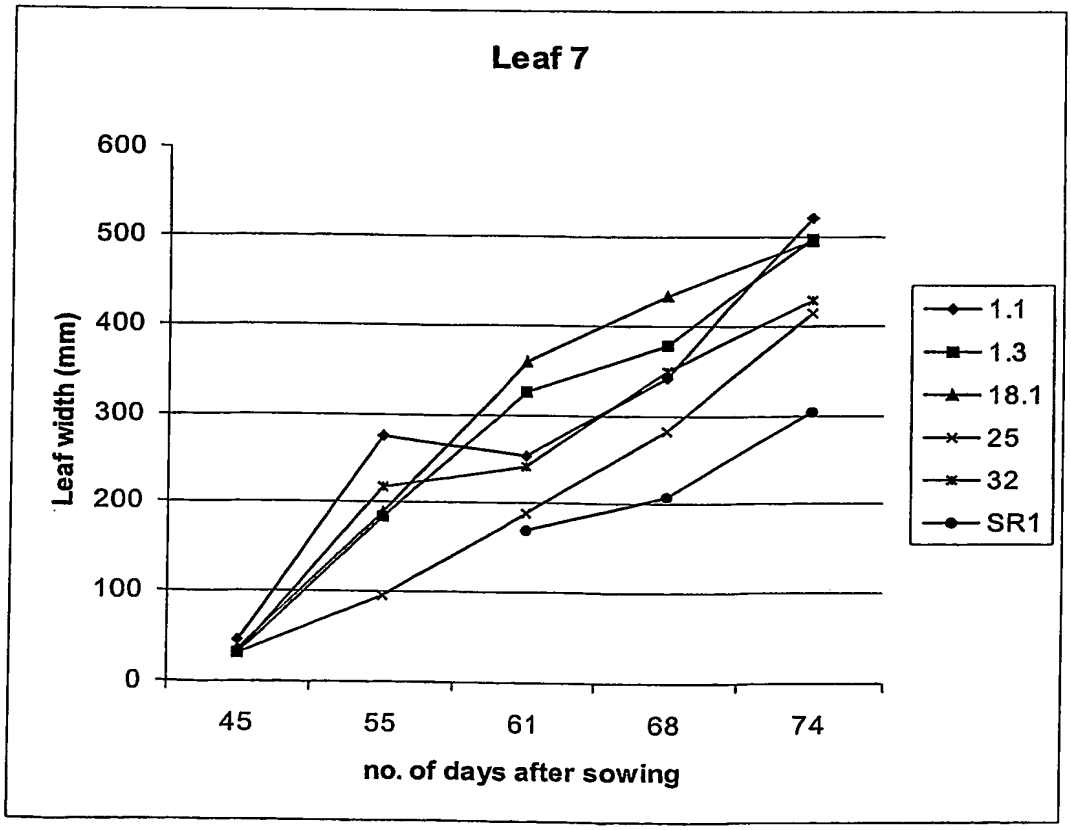

FIGURE 5 C - D

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FIGURE 6

SEQ ID NO 2 protein Arabidopsis thaliana cdc27A1

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FIGURE 6 (CONTINUED)

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Universidade Federal do Rio de Janeiro

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Phe Pro Ser Glu Val Asn Leu Gln Leu Leu Ala Arg Cys Tyr Leu Ser
35 40 45

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50 55 60

Pro Gln Ser Arg Tyr Leu Phe Ala Phe Ser Cys Phe Lys Leu Asp Leu
65 70 75 80

Leu Gly Glu Ala Glu Ala Ala Leu Leu Pro Cys Glu Asp Tyr Ala Glu
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Leu Lys Ile Met Gln Gln Pro Gly Asp Ile Pro Pro Asn Thr Asp Arg
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Glu Ser Gly Arg Arg Arg Ser Ala Arg Ile Ala Ala Arg Lys Lys Asn
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Pro Met Ser Gln Ser Phe Gly Lys Asp Ser His Trp Leu His Leu Ser
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Pro Ser Glu Ser Asn Tyr Ala Pro Ser Leu Ser Ser Met Ile Gly Lys
                325                 330                 335

Cys Arg Ile Gln Ser Ser Lys Glu Val Ile Pro Asp Thr Val Thr Leu
            340                 345                 350

Asn Asp Pro Ala Thr Thr Ser Gly Gln Ser Val Ser Asp Ile Gly Ser
        355                 360                 365

Ser Val Asp Asp Glu Glu Lys Ser Asn Pro Ser Glu Ser Ser Pro Asp
    370                 375                 380

Arg Phe Ser Leu Ile Ser Gly Ile Ser Glu Val Leu Ser Leu Leu Lys
385                 390                 395                 400

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465                 470                 475                 480

Glu Glu Met Arg Leu Gly Tyr Leu Ala Gln Glu Leu Ile Ser Val Asp
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Arg Leu Ser Pro Glu Ser Trp Cys Ala Val Gly Asn Cys Tyr Ser Leu
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Arg Lys Asp His Asp Thr Ala Leu Lys Met Phe Gln Arg Ala Ile Gln
        515                 520                 525

Leu Asn Glu Arg Phe Thr Tyr Ala His Thr Leu Cys Gly His Glu Phe
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Ala Ala Leu Glu Glu Phe Glu Asp Ala Glu Arg Cys Tyr Arg Lys Ala
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Thr Tyr Leu Arg Gln Glu Lys Phe Glu Phe Ala Gln His Gln Phe Gln
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Tyr Lys Ala His Ile Leu Thr Ser Leu Gly Asp Tyr His Lys Ala Gln
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His Ala Ser Leu Gly Lys Ile Tyr Asn Gln Leu Lys Gln Tyr Asp Lys
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